vaccinations against malaria, the control of this disease is heavily dependent upon the use of antimalarial drugs. Antimalarial drugs, such as methylene blue, are effective therapies against human malaria. At a specific concentration, methylene blue has been shown to be a selective inhibitor of the parasite's glutathione reductase (*Pf*GR).
Glutathione reductase is an important target when studying malaria drug resistance because it is a flavoenzyme that regenerates glutathione, which is an essential protein for antioxidant defense against cell damage. Methylene blue is also a substrate that is reduced by gluthathione reductase to produce leucoMB. This is then spontaneously oxidized by molecular oxygen to form methylene blue again. During this process, reactive oxygen species, such as hydrogen peroxide and superoxide form. These act as recycling catalysts against infectious organisms. Due to *Pf*GR's central position in redox control, it is ranked number one as an antimalarial drug target. The goal of this research is to study the interface between methylene blue and the putative protein target, glutathione reductase, in order to understand the drug action mechanism. *Pf*GR was expressed and purified and hydrogen-deuterium exchange (HDX) will be used to map the drug-protein interface. We will present the mass spectrometry data for the solvent exposed peptides after digestion with pepsin when *Pf*GR is complexed with methylene blue, which will allow us to narrow down the active site.

279-Pos Board B59
Effects of Molecular Crowding on the Binding Affinity of Dihydrofolate for Dihydrofolate Reductase
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The reduction of dihydrofolate (DHF) to tetrahydrofolate (THF) by the enzyme dihydrofolate reductase (DHFR), using NADPH as a cofactor, is an essential part of the folate cycle. The inhibition of DHFR leads to interruption of DNA synthesis and consequently cell death, making this enzyme a crucial target in the treatment of cancer and other diseases. Previous studies examined the effects of small molecule osmolytes on the substrate interactions with two non-homologous DHFRs, E. coli chromosomal DHFR (EcDHFR) and R67 DHFR, with vastly different active site structures. The results indicated that DHF weakly interacts with the osmolytes in solution, shifting the binding equilibrium from DHF bound to DHFR to unbound DHF. It is hypothesized that similar weak, nonspecific interactions may also occur between cellular proteins and DHF. Weak interactions between cellular proteins and DHF would have consequences in vivo, where the concentration of the cellular milieu is approximately 300 g/L. Under the crowded conditions in the cell, there is a higher propensity for intermolecular interaction.
Crowding effects of macromolecules in concentrations similar to those in vivo were examined. Isothermal titration calorimetry (ITC) and enzyme kinetic assays were used to detect effects of molecular crowders by monitoring activity of the (DHFR)-NADPH or DHF complex and the ternary DHFR-DHF-NADPH complex in the presence of these crowders. To recreate the conditions of molecular crowding in vivo, the binding of the enzyme-ligand complexes in the presence of molecular weight crowding agents (lysozyme or casein) was examined. Analysis of the Kd's and Km's indicated a correlation between increased molecular crowding in the solution and weakened binding of the DHFR-substrate complexes. These findings indicate an importance of molecular crowding on EcDHFR activity in vivo.

280-Pos Board B60
Quantifying the Influence of the Crowded Cytoplasm on Small Biomolecule Diffusion via Homogenization Theory
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Cytosolic crowding is known to influence the thermodynamics and kinetics of in vivo chemical reactions. Crowders, including proteins, macromolecular assemblies and intracellular organelles, reduce the volume available to a diffusing substrate and thereby lower its effective diffusion constant relative to its rate in bulk solution. However, the nature of a substrate's interaction with crowders, such as through electrostatic or van der Waals forces, can further influence the effective diffusion rate. To probe the impact of crowding over micron-scale intracellular distances, we apply a multi-scale mathematical theory, homogenization, to estimate effective diffusion rates for ions and small biomolecules diffusing in a densely-packed lattice of representative cytosolic proteins. Specifically, via the finite element method we numerically solve the homogenized diffusion equation for a nearly 1 micron cubed cytosolic fraction based on published Brownian dynamics data of the bacterial cytoplasm (McGuffee and Elcock, PLOS Computational Biology, vol. 6, no. 3, p. e1000694, Mar. 2010). Our simulations quantify how the crowded volume fraction, irregularity of protein shapes and distribution, and molecular interactions influence the diffusion rates of small molecules.

281-Pos Board B61
Specific or General - It is All About Solute Interactions with the Pore
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Recently, Kojima and Nikaido (PNAS, 110: E2629, 2014) examined the idea of potential specificity of porin channels, where -lactam antibiotics' interaction with the pore-lining residues, demonstrated *in vitro*, was suggested to facilitate transmembrane transport (PNAS, 99:9789, 2002). The authors came to a conclusion that the binding inside OmpF channel does not significantly affect the penetration of ampicillin and benzylpenicillin.
While we emphatically agree with the authors that attractive interactions *per se* do not constitute the leading imperative in search for an antibiotic with the ''magic bullet'' potential, it would be surprising if Nature (or pharmaceutical companies, by trial and error) had not explored the benefits of these interactions to facilitate antibiotic translocation. Indeed, the probability of translocation through the OmpF pore for the molecule of ampicillin size that is already at the channel entrance could be estimated as a fraction of one percent (JCP, 116:9952, 2002). This is an impressively small number.
The presence of optimal attractive interactions is able to compensate for the entropic cost of confinement (Biochemistry, 52:9246, 2013) and thus increase the translocation probability to 0.5, its maximum value for passive, although interaction-assisted, diffusion. Certainly, not every attractive interaction is optimal or even beneficial for translocation. Too strong or wrongly distributed binding can be detrimental. However, attractive interactions in a particular channel-solute pair are able, at least in principle, to transform a porin, which is ''general'' for many other solutes, into a ''specific'' one for the particular pair.

282-Pos Board B62
Using Sedimentation Velocity to Investigate the Nucleotide-Linked Assembly of E. coli ClpA
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The AAA+ (ATPases Associated with various cellular Activities) group of proteins is a large superfamily whose members are present in single-celled and multi-celled organisms. This family is subdivided into two classes; Class I ATPases contain two ATPase domains per monomer while Class II ATPases contain one ATPase domain per monomer. Both classes utilize ATP binding and hydrolysis to undergo conformational changes that allow them to perform mechanical work. Further, the biologically active form for most members of the AAA+ family is a hexamer. Quantitatively examining the biologically-relevant activities for AAA+ proteins requires a precise determination of the concentration of hexamers in solution. The difficulty in determining the hexamer concentration is that the oligomeric state of interest often resides in a dynamic equilibrium. This leads to a distribution of oligomeric states in solution. Therefore, insight into nucleotide-linked assembly is required to be able to understand how any of the AAA+ motors function. ClpA is a Class I AAA+ protein. Apparent self-association equilibrium constants were obtained using sedimentation velocity and were subsequently analyzed using binding polynomials that incorporate either zero cooperativity or infinite cooperativity into the nucleotide binding process to yield apparent nucleotide binding constants. Here we show that the population of ClpA dimers, tetramers, and hexamers is dependent upon nucleotide concentration. Further, apparent nucleotide binding constants were found for each oligomeric state. The above-stated information was used to generate a prediction of ClpA hexamer population that takes into account nucleotide-linked assembly. We anticipate the results of this study will set the stage for being able to predict the concentration of hexamers at any given nucleotide concentration, therefore allowing us to probe the kinetic and energetic properties of ClpA as well as other members of the AAA+ superfamily.

283-Pos Board B63
Macromolecular Crowder and Ligand Compete for the Closed Domain Cleft of Maltose Binding Protein
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Protein-ligand binding is central to many biochemical processes including enzyme catalysis/inhibition and cellular signaling. In the cellular context, these processes occur in milieus crowded with bystander macromolecules. Growing evidence suggests that the macromolecular crowders are not inert but influence the biochemical processes. A previous study of our lab demonstrated that a synthetic polymer crowder, Ficoll70, and a ligand, maltose, compete for binding with the maltose binding protein (MBP), a periplasmic protein involved in nutrient uptake and chemotaxis [Miklos and Zhou, PLoS ONE 8, e74969 (2013)]. Fluorescence and NMR spectroscopy showed that Ficoll70 weakly binds to MBP and this